LEAF-TISSUE PRODUCTION AND WATER CONTENT IN A MUTANT RACE OF PHASEOLUS VULGARIS

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Introductory

In a preceding paper[1] it was shown that the survival of the bean plant is in a measurable degree dependent upon the morphological characteristics of the seedling. In 1915 a series of investigations was undertaken to determine, if possible, something of the proximate causes of the differential death rate. It was also hoped that some light would thereby be thrown upon the proximate causes underlying the occurrence of teratological variations in the seedlings of *Phaseolus*. In undertaking this work the assumption seemed justified that if innate physiological conditions which might affect growth be associated with morphological variations, some influence of these factors should be recorded in the size or other characteristics which result from the relatively enormous expansion which the organs of the embryo undergo in the course of germination and the establishment of the seedling.

A first study[2] demonstrated that teratological seedlings in general show a lower capacity for the development of primordial leaf tissue than do normal seedlings grown under as nearly as possible identical conditions. The data then available indicated that a reduction of the volume of primordial leaf tissue is associated with abnormalities of all the abnormal types studied, but that the type of variation influences in some degree the amount of reduction. In these first experiments the conclusions were based on primordial leaves only. The use of such leaves has the obvious disadvantage that they are formed in the seed, and undergo merely an enormous expansion (and possibly a little differentiation) in the germination

[1] HARRIS, J. ARTHUR, A simple demonstration of the action of natural selection. Science, N.S. **36**:713–715. 1912.

[2] ———, Studies on the correlation of morphological and physiological characters: The development of the primordial leaves in teratological bean seedlings. Genetics **1**:185–196. 1916.

of the seed and the development of the plantlet to the stage at which measurements were made. Since the development of the primordial leaves during the germination and establishment of the seedling is relatively great, it seemed quite legitimate to use the weight of green tissue produced by these leaves as a measure of the physiological capacity of seedlings of various types. The fact that these leaves are differentiated in the seed, however, constitutes a valid objection against their use as a sole measure of the physiological capacity of the seedling. For such purposes a constant based upon some organ developed later seemed desirable.

In a second study,[3] therefore, the tissue weight determinations were extended to the trifoliate leaves of the third node, as well as to the primordial leaves of the second node. This leaf was used because groups of plants of more uniform development can be selected at the time of maturity of this leaf, than at any later stage, and because the first compound leaf reaches a degree of maturity sufficient for the purpose of the present study before the primordial leaves are too old to be used. It is possible, therefore, to check results by determinations made on organs differentiated both in age and in structure. In the first investigation the green weight of the leaf tissue served as the fundamental measurement. In addition to this character certain measurements on the sap properties were also made. In the study of the saps some difficulties were encountered, and it seemed desirable to discontinue that phase of the work temporarily and to carry out determinations of dry weight and water content instead. The present study, therefore, has to do only with the green weight, the dry weight, and the percentage of dry matter.

Recent investigations fell into two phases. The first was an endeavor to determine to what extent seedlings which are morphologically aberrant in the race to which they belong also differ from the normal seedling of the race in their physiological characters, in so far as these can be measured by the capacity for the production of tissue. In the second the investigation was extended from intra-racial to inter-racial comparisons, to ascertain if possible to what

[3] HARRIS, J. ARTHUR, Further studies on the interrelationship of morphological and physiological characters in seedlings of *Phaseolus*. Brooklyn Bot. Gard. Mem. 1:167–174. 1918.

extent a highly abnormal race differs from the parental strain from which it originated.

Materials and methods

In this paper the characteristics of a fully heritable teratological race are considered. The material was furnished by a tetracotyledonous race, the origin and general characteristics of which have been considered elsewhere.[4] The tissue of plants of the tetracotyledonous race were compared with those of the normal line from which it originated.

Seeds of the two series grown in the same field in 1917 were germinated in flats of sand in 1919. Four lots of fifty seeds each, two of the tetracotyledonous plants and two of the normal ancestral line, were germinated in alternate positions in the same flat. Conditions, therefore, were as nearly comparable as possible in the germination of the two series. When the seedlings were of the proper size for potting, one seedling of the tetracotyledonous race and one normal control taken from the same flat were transferred to 3-inch pots of soil, where they stood until they were ready for the collection of samples of tissues. Weighings were then made of the primordial leaves in the two cases. Thus, although weight and other characteristics vary from sample to sample because of age and the innumerable slight influences of significance in growth, the aberrant plants and their controls from the very beginning had as nearly as possible identical environment. However much the pairs combined in the same sample may differ among themselves, there seems no possibility of considering that the differentiation here shown to exist between the morphologically typical and the morphologically aberrant individuals is due to any extrinsic cause. In the absence of any knowledge of the amount of variation in the characteristics of the leaves to be investigated, it was impossible to compute in advance the size of the sample which should be taken. Accordingly it was arbitrarily fixed as 100 plants.[5]

[4] HARRIS, J. ARTHUR, A tetracotyledonous race of *Phaseolus vulgaris*. Mem. N.Y. Bot. Gard. **6**:229–244. 1916.

———, De Vriesian mutation in the garden bean *Phaseolus vulgaris*. Nat. Acad. Sci. **2**:317–318. 1916.

[5] Sample 305 contained 96 plants, sample 252 only 81 plants, and sample 259 contained 138 plants.

In work with the variants in normal lines of beans there is no difficulty whatever in distinguishing primordial leaves from those subsequently formed, except occasionally in extreme variations involving stem characters such as would ordinarily be classed as fasciations. In the case of the tetracotyledonous race, however, it is often difficult to distinguish between true primordials (those formed in the seed) and the simple leaves (not compound) formed subsequently. This difficulty was noted in the first paper on the tetracotyledonous race, and two series of countings at different stages of development of the seedling were made to determine to what extent personal equation may affect the constants for number of primordial leaves.

For practical reasons it was not feasible to count the leaves of the tetracotyledonous plants used in these experiments immediately after germination. Countings, therefore, were made just before the samples were taken. The numbers recorded are those of leaves which were regarded as certainly primordial. Those which from their color or texture appeared to be of subsequent development were omitted. In this race filaments are of rather frequent occurrence. These are probably morphologically much reduced leaves, and were also disregarded. Thus the number of primordial leaves is probably on the average slightly under rather than over the true number for the series as a whole. Since we are primarily concerned with a comparison between definite types of seedling classification with respect to number of leaves, this procedure can introduce no sensible error into the results.

Because of some uncertainty as to the leaves which were to be considered primordial and the considerable variation in the stages of development of the compound leaves in the tetracotyledonous plants, it did not seem feasible in the majority of determinations to consider separately the weight of tissue formed by the compound leaves. This, however, has been done indirectly in the case of certain samples based on the plants as a whole.

Data

The data fall in three groups: a series of weighings of primordial leaves of plants unclassified with respect to number of primordial leaves; a series of weighings of primordial leaves of plants classified

with respect to number of primordial leaves; and a series of weighings of total epicotyledonary tissue.

TABLE I

MEAN GREEN WEIGHT PER PLANT AND PER LEAF IN SEEDLINGS OF A TETRACOTYLEDONOUS RACE AND IN NORMAL PLANTS OF THE ANCESTRAL RACE

SAMPLE	VALUES PER PLANT				VALUES PER LEAF			
	Abnormal	Control	Difference	Percentage difference	Abnormal	Control	Difference	Percentage difference
Unclassified								
226.....	0.6991	0.7516	−0.0525	− 6.9	0.1718	0.3758	−0.2040	−54.2
227.....	0.6972	0.7607	−0.0635	− 8.3	0.1680	0.3804	−0.2124	−55.8
228.....	0.6323	0.7568	−0.1245	−16.4	0.1542	0.3784	−0.2242	−59.2
229.....	0.7012	0.6862	+0.0150	+ 2.1	0.1793	0.3431	−0.1638	−47.7
2 leaves								
258.....	0.4520	0.7263	−0.2743	−37.7	0.2260	0.3632	−0.1372	−37.7
305.....	0.5958	0.7994	−0.2036	−25.4	0.2979	0.3997	−0.1018	−25.4
3 leaves								
255.....	0.6313	0.7760	−0.1447	−18.6	0.2104	0.3880	−0.1776	−45.7
273.....	0.5662	0.7189	+0.1527	−21.2	0.1887	0.3595	−0.1708	−47.5
283.....	0.5791	0.7766	−0.1975	−25.4	0.1930	0.3883	−0.1953	−50.2
303.....	0.6577	0.8015	−0.1438	−17.9	0.2192	0.4008	−0.1816	−45.3
319.....	0.6200	0.7817	−0.1617	−20.6	0.2067	0.3909	−0.1842	−47.1
4 leaves								
253.....	0.6703	0.7786	−0.1083	−13.9	0.1676	0.3893	−0.2217	−56.9
264.....	0.5994	0.6671	−0.0677	−10.1	0.1499	0.3336	−0.1837	−55.0
286.....	0.7066	0.8103	−0.1037	−12.7	0.1767	0.4052	−0.2285	−56.3
296.....	0.6556	0.8142	−0.1586	−19.4	0.1639	0.4071	−0.2432	−59.7
298.....	0.7368	0.9028	−0.1660	−18.3	0.1842	0.4514	−0.2672	−59.1
314.....	0.7201	0.7783	−0.0582	− 7.4	0.1800	0.3892	−0.2092	−53.7
320.....	0.7375	0.7529	−0.0154	− 2.0	0.1844	0.3765	−0.1921	−51.0
5 leaves								
254.....	0.6371	0.7639	−0.0998	−13.5	0.1274	0.3685	−0.2411	−65.4
268.....	0.6409	0.6987	−0.0578	− 8.2	0.1282	0.3494	−0.2212	−63.3
287.....	0.7334	0.7867	−0.0533	− 6.7	0.1467	0.3934	−0.2467	−62.7
300.....	0.8032	0.8366	−0.0334	− 3.9	0.1606	0.4183	−0.2577	−61.6
321.....	0.7125	0.7987	−0.0862	−10.7	0.1425	0.3994	−0.2569	−64.3
6 leaves								
256.....	0.7123	0.7268	−0.0145	− 1.9	0.1187	0.3634	−0.2447	−67.3
289.....	0.7351	0.7646	−0.0295	− 3.8	0.1225	0.3823	−0.2598	−67.9
307.....	0.7950	0.8248	−0.0298	− 3.6	0.1325	0.4124	−0.2799	−67.8
7 leaves								
259.....	0.7312	0.7785	−0.0473	− 6.0	0.1045	0.3893	−0.2848	−73.1
Epicotyl								
244.....	1.4388	2.0369	−0.5981	−29.4	.....	.....	.....	.....
246.....	1.5442	1.9133	−0.3691	−19.3	.....	.....	.....	.....
248.....	1.5448	2.0396	−0.4948	−24.3	.....	.....	.....	.....
250.....	1.6325	1.9028	−0.2703	−14.2	.....	.....	.....	.....
252.....	1.0634	1.1512	−0.0878	− 7.6	.....	.....	.....	.....

PLANTS UNCLASSIFIED WITH RESPECT TO NUMBER OF PRIMORDIAL LEAVES.—In preliminary work (samples 226–229) the total weight of primordial leaf tissue in the abnormal seedlings is compared

with the total weight in the control plants irrespective of the number of primordial leaves formed by the individual plants of the tetracotyledonous race. The total number of leaves per plant, however, was determined in these four series.[6] Thus it is possible to give the average weights both per plant and per leaf in the two series. The results show that in three of the four cases the green weight as given in table I of the approximately four primordial leaves of the tetracotyledonous race is lower than that of the two primordial leaves of the dicotyledonous strain. The percentage differences in total weight range from +2.1 to −16.4, with a general average of −7.37. When the comparison is made on the basis of mean weight per leaf, the primordial leaf of the abnormal seedling is found to be on the average 54.22 per cent lighter than the leaf of the normal seedling.

For dry weight, given in table II, all four series show lower average weight in the tetracotyledonous strain. The percentage differences for dry weight of primordial leaves per plant vary from −1.6 to −18.0, with a general average of −10.90. On the basis of mean dry weight per leaf, the weight for tetracotyledonous plants is found to be from 49.6 to 59.9 per cent lower than that of the normal seedling, with a general average percentage difference of −55.92. Thus the results for these four samples clearly indicate that an abnormal race shows the same relationship to the normal parental race as do abnormal individual seedlings to the normal seedlings in the same race.

PLANTS CLASSIFIED WITH RESPECT TO NUMBER OF PRIMORDIAL LEAVES.—Upon the completion of this preliminary comparison it seemed worth while to analyze the relationships more minutely by considering individually the results for seedlings of the tetracotyledonous race with varying numbers of primordial leaves. These results were only attained at the cost of great labor, since it was difficult to secure considerable numbers of seedlings of any given type simultaneously. It was necessary, therefore, to make determinations for abnormal and control plants in small subsamples, and to combine these to form samples of 100 seedlings

[6] The average numbers per plant were as follows in the four samples: 226=4.07, 227=4.15, 228=4.10, and 229=3.91.

each. The results are shown in table I for green weight, table II for dry weight, and in table III for the percentage of dry matter in the primordial leaves. The data show that in the case of both green and dry weight tissue production is invariably higher in the

TABLE II

MEAN DRY WEIGHT PER PLANT AND PER LEAF IN SEEDLINGS OF A TETRACOTYLEDONOUS RACE AND IN NORMAL PLANTS OF THE ANCESTRAL RACE

SAMPLE	VALUES PER PLANT				VALUES PER LEAF			
	Abnormal	Control	Difference	Percentage difference	Abnormal	Control	Difference	Percentage difference
Unclassified								
226.....	0.0529	0.0600	−0.0071	−11.8	0.0130	0.0300	−0.0170	−56.6
227.....	0.0530	0.0604	−0.0074	−12.2	0.0128	0.0302	−0.0174	−57.6
228.....	0.0528	0.0644	−0.0116	−18.0	0.0129	0.0322	−0.0193	−59.9
229.....	0.0539	0.0548	−0.0009	− 1.6	0.0138	0.0274	−0.0136	−49.6
2 leaves								
258.....	0.0355	0.0587	−0.0232	−39.5	0.0178	0.0294	−0.0116	−39.4
305.....	0.0403	0.0542	−0.0139	−25.6	0.0202	0.0271	−0.0069	−25.4
3 leaves								
255.....	0.0492	0.0649	−0.0157	−24.1	0.0164	0.0325	−0.0161	−49.5
273.....	0.0427	0.0562	−0.0135	−24.0	0.0142	0.0281	−0.0139	−49.4
283.....	0.0404	0.0565	−0.0161	−28.4	0.0135	0.0283	−0.0148	−52.2
303.....	0.0404	0.0539	−0.0135	−25.0	0.0135	0.0270	−0.0135	−50.0
319.....	0.0360	0.0488	−0.0128	−26.2	0.0120	0.0244	−0.0124	−50.8
4 leaves								
253.....	0.0517	0.0641	−0.0124	−19.3	0.0129	0.0321	−0.0192	−59.8
264.....	0.0470	0.0532	−0.0062	−11.6	0.0118	0.0266	−0.0148	−55.6
286.....	0.0493	0.0576	−0.0083	−14.4	0.0123	0.0288	−0.0165	−57.2
296.....	0.0409	0.0540	−0.0131	−24.2	0.0102	0.0270	−0.0168	−62.2
298.....	0.0444	0.0568	−0.0124	−21.8	0.0111	0.0284	−0.0173	−60.9
314.....	0.0444	0.0516	−0.0072	−13.9	0.0111	0.0258	−0.0147	−56.9
320.....	0.0434	0.0478	−0.0044	− 9.2	0.0109	0.0239	−0.0130	−54.3
5 leaves								
254.....	0.0488	0.0604	−0.0116	−19.2	0.0098	0.0302	−0.0204	−67.5
268.....	0.0462	0.0537	−0.0075	−13.9	0.0092	0.0269	−0.0177	−65.7
287.....	0.0501	0.0562	−0.0061	−10.8	0.0100	0.0281	−0.0181	−64.4
300.....	0.0496	0.0549	−0.0053	− 9.6	0.0099	0.0275	−0.0176	−64.0
321.....	0.0423	0.0500	−0.0077	−15.4	0.0085	0.0250	−0.0165	−66.0
6 leaves								
256.....	0.0528	0.0558	−0.0030	− 5.3	0.0088	0.0279	−0.0191	−68.4
289.....	0.0520	0.0555	−0.0035	− 6.3	0.0087	0.0277	−0.0190	−68.5
307.....	0.0496	0.0565	−0.0069	−12.2	0.0083	0.0283	−0.0200	−70.6
7 leaves								
259.....	0.0557	0.0614	−0.0057	− 9.2	0.0080	0.0307	−0.0227	−73.9
Epicotyl								
244.....	0.1048	0.1507	−0.0459	.....	.....	.....	.....	.....
246.....	0.1159	0.1443	−0.0284	.....	.....	.....	.....	.....
248.....	0.1127	0.1514	−0.0387	.....	.....	.....	.....	.....
250.....	0.1193	0.1413	−0.0220	.....	.....	.....	.....	.....
252.....	0.0939	0.1045	−0.0106	.....	.....	.....	.....	.....

two primordial leaves of the normal ancestral strain than it is in the two to seven leaves of the tetracotyledonous strain. The percentage

TABLE III

PERCENTAGE DRY SUBSTANCE IN SEEDLINGS OF A TETRACOTYLEDONOUS RACE AND IN NORMAL PLANTS OF THE ANCESTRAL RACE

SAMPLE	PRIMORDIAL LEAVES		
	Abnormal	Control	Difference
Unclassified			
226	7.566	7.982	−0.416
227	7.601	7.940	−0.339
228	8.350	8.509	−0.159
229	7.686	7.986	−0.300
2 leaves			
258	7.853	8.082	−0.229
305	6.765	6.788	−0.023
3 leaves			
255	7.793	8.363	−0.570
273	7.541	7.817	−0.276
283	6.976	7.275	−0.299
303	6.142	6.724	−0.582
319	5.806	6.242	−0.436
4 leaves			
253	7.712	8.232	−0.520
264	7.841	7.974	−0.133
286	6.977	7.108	−0.131
296	6.238	6.632	−0.394
298	6.026	6.291	−0.265
314	6.165	6.629	−0.464
320	5.884	6.348	−0.464
5 leaves			
254	7.659	8.196	−0.537
268	7.208	7.685	−0.477
287	6.831	7.143	−0.312
300	6.175	6.562	−0.387
321	5.936	6.260	−0.324
6 leaves			
256	7.412	7.677	−0.265
389	7.073	7.258	−0.185
307	6.238	6.850	−0.612
7 leaves			
259	7.620	7.892	−0.272
Epicotyl			
244	7.283	7.398	−0.115
246	7.505	7.541	−0.036
248	7.295	7.423	−0.128
250	7.307	7.425	−0.118
252	8.834	9.083	−0.249

values show considerable variation from sample to sample. As might have been expected on a priori grounds, the deficiency of

the weight of primordial leaves in the tetracotyledonous line is greatest when only two leaves are formed.

A comparison of the average percentage differences for abnormal plants with various numbers of leaves gives the following results:

No. of leaves	Green weight	Dry weight
2	−31.55	−32.55
3	−20.74	−25.54
4	−11.97	−16.34
5	− 8.60	−13.78
6	− 3.10	− 7.93

Only one sample is available for seedlings with seven primordial leaves, and it is omitted from the comparison. The results for the other five classes show that:

a) The difference between the total weight of primordial leaf tissue in the abnormal seedling and its normal control decreases as the number of leaves in the abnormal plant increases, but that throughout the entire range of variation of leaf number studied the tetracotyledonous plant produces a smaller total weight of leaf tissue than do normal plants of the line from which it was derived.

b) The differences between tetracotyledonous and dicotyledonous plants are always greater when the comparison is made on the basis of dry weight than when it is made on the basis of green weight.

If the comparison be made on the basis of average weight per leaf the following results are obtained:

No. of leaves	Green weight	Dry weight
2	−31.55	−32.40
3	−47.16	−50.38
4	−55.95	−58.12
5	−63.46	−65.52
6	−67.66	−69.16

The percentage differences in average weight per leaf of course increase as the number of leaves in the abnormal seedlings increases. Again the greater percentage difference when dry weight serves as a basis of comparison is conspicuous. The percentage of dry matter

in the seedlings of this extremely abnormal race is shown in comparison with the normal control plants in table III. The results are self-explanatory. Without exception, in the twenty-three samples representing weighings of 9958 leaves of abnormal and 4668 leaves of normal plants, the percentage of dry matter is lower in the abnormal than in the control series. A study of the averages for the individual groups of seedlings, classified with respect to primordial leaf number, does not suggest a significant difference in the percentage of dry matter in the different classes of seedlings. Probably a far larger series of weighings would be required to bring out such a differentiation if it exists at all.

TOTAL WEIGHT OF EPICOTYLEDONARY TISSUE IN PLANTS OF TERATOLOGICAL AND NORMAL RACES.—In the foregoing discussion comparisons were limited to the weight of primordial leaves. This was done because of the difficulty of securing leaves subsequently formed in comparable stages of development in the normal and teratological seedlings. It seemed desirable to supplement these studies by the determination of the total weight of tissue produced by the two races. The results for a comparison of the total weight of tissue produced above the cotyledonary node by tetracotyledonous plants and their normal control of line 139, are shown under the heading "epicotyl" in the fundamental tables of data.

The constants show that without exception the green weight and dry weight per plant and percentage dry matter are higher in the normal than in the tetracotyledonous plants. The percentage differences range from −7.6 to −29.4 in the case of green weight, and from −10.1 to −30.5 in the case of dry weight. The differences in percentage of dry matter range from −0.036 to −0.249. The average weight of green tissue per plant is 1.4447 for the abnormal and 1.8088 for the control series, or a difference of −0.3640 gm. The average dry weight per plant is 0.1093 for the abnormal and 0.1384 for the normal seedling, or a difference of −0.0291 gm. The average percentage difference for the green weight is −18.96, while for dry weight the difference is −20.30. The percentage of dry material in the abnormal seedling is 7.6448 as compared with 7.7740 in the control, a difference of −0.1292.

Summary

This paper presents the results of an investigation of green weight, dry weight, and of the ratio of green weight to dry weight in primordial leaf tissue in mutant and parental races of *Phaseolus vulgaris*. The data show that when grown under as nearly identical conditions as possible the primordial leaves of the mutant (tetracotyledonous) show a smaller green weight, a smaller dry weight, and a lower ratio of dry weight to green weight than those of the normal (dicotyledonous) parental race. Thus the tetracotyledonous race is distinguished not merely by striking morphological differences, but by physiological differentiation as well. In this respect the results for the heritable mutant race are in agreement with those for variant individuals within the same strain.

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